REMARKS

The Office Action dated June 1, 2006 has been received and carefully reviewed. The undersigned counsel wishes to thank the Examiner for the telephonic interview of October 5, 2006.

Claims 94 and 95 have been withdrawn in view of the Restriction Requirement being made final. Claims 87-90 have been amended to depend from claim 91 as suggested by the Examiner during the interview.

Rejection Under 35 U.S.C. §112, first paragraph (new matter)

The Examiner rejected claims 84-94 under 35 U.S.C. §112, first paragraph, for failing to comply with the written description requirement. In particular, the Examiner contends that the claims, as amended, contain subject matter that was not described in the original specification in such a way as to reasonably convey to one of skill in the art that the inventor had possession of the invention at the time of filing. According to the Examiner, claim 84 contains definitions and formulas, such as Formula II through Formula IV which are not supported in the originally filed disclosure. Applicants respectfully traverse this rejection.

The undersigned counsel and the Examiner conducted a telephonic interview on October 5, 2006. The Examiner indicated that page number support and explanation of the schematic representations of Formulas I- IV as in claims 84-95 was necessary, because the specification does not describe the formulas and diagrams in the claims explicitly. Applicants have supplied the Examiner with five pages of figures which schematically explain the

Applicants' invention and the derivation of the Formulas in claim 84. These pages are included with this Response as Attachment A, and further described below. Support for the description of the general process is found at page 13, line 28 to page 18, line 12 of the specification.

In page 1/5 of Attachment A, Figure 1 depicts the OspC antigenic determinant peptide. A critical characteristic of the OspC antigen is that it must be presented in a C-terminal orientation to bind to antibodies, which is not how standard peptides are presented for antibody binding. In Figure 2, a lysine tree is shown from the J.P. Tam reference. The Tam reference teaches a method of generating many N-terminal presenting peptides. Figure 3 shows the same Tam prior art method of multiple antigen presentation, known as the MAP system, using the OspC sequences. The MAP system is limited to N-terminal presentation of peptides, therefore it cannot be used to bind antibodies specific for C-terminal ends of the antigenic peptide.

On page 2/5 of Attachment A, Figure 4 illustrates the problem being solved by Applicants' invention, namely presentation of peptide antigens in a C-terminal orientation, instead of an N-terminal orientation. Figure 5 shows what the desired multiple antigen presentation would look like. There, two OspC peptides are linked at their N-terminals and have free C-terminal ends able to bind to immune response receptors.

Page 3/5 of Attachment A is a schematic illustration of the method of Applicants' invention. Figure 6, top, depicts a resin bead in a solid-state synthesis of a desired antigen peptide after completion of the synthesis of the peptide (See, the

specification at page 18, line 30 - Page 21, line 20; page 41, line 5 - page 47, line 27). It is during this step, that measurement of the amount of substitution of peptides (mole/g resin) is performed to identify how many moles of dicarboxylic acid must be added to obtain a 1:1 mixture of acylated and un-acylated peptide chains. Two standard methods for measuring the substitution are provided in the specification, in the Examples section entitled, "Estimation of the coupling yield of the first N-α-amino protected amino acid" (page 45, lines 13-23).

Figure 6 shows that the synthesized peptides are attached to the resin bead at the C-terminal end, and the N-terminal ends of the peptides are free. In Figure 6, at the bottom, the peptide chains are shown as acylated with 0.5 equivalents of an achiral dicarboxylic acid represented by $XN(CH_2COOH)_2$, so that approximately half of the free α-amino groups of the fully side-chain protected peptide chains, react with the achiral dicarboxylic acid. Again, the dicarboxylic acid groups should be achiral because this is the only way to prevent racemic mixtures of peptides.

On page 4/5 of Attachment A, Figure 7 illustrates acylation of the remaining free N-terminal ends in step 2 of the process of the claimed invention, with the free carboxylic acid group now present on the other half of the bound peptide chains. The dicarboxylic acids are activated, and the reaction of the free COOH group with a free N-terminal of the remaining peptides results in a cyclization of two peptides with a dicarboxylic acid group as shown in Figure 7, bottom. (Page 20, lines 20-33; Page 21, line 21 - Page 22, line 30)

On page 5/5 of Attachment A, Figure 8 shows the next step

in the claimed process. Here the cyclized di-peptides from Figure 7 are cleaved from the resin bead using the standard Fmoc deprotection and cleavage methods used in solid phase peptide synthesis. The result is the LPA molecule claimed having two peptides linked at their N-terminal ends by an amino group, and having their C-terminal ends free (Page 23, lines 1-30).

Applicants have supplied the foregoing support for the amended claims as requested by the Examiner. Applicants submit that it would be clear to one of ordinary skill in the art that the claim language is fully supported by the originally filed specification.

Rejection under 35 U.S.C. §112, first paragraph (written description)

The Examiner rejected claims 84-94 under 35 U.S.C. 112, first paragraph, because the Examiner deemed that the specification, while being enabling for the antigenic peptide sequence of Borrelia burgdorferi and iminodiacetic acid as the bridging group, the specification does not reasonably provide enablement for a claimed genus of ligand presenting assembly comprising any 4-20 amino acid peptide chain or its homologs or mimics, with any achiral dicarboxylic acid as a bridging group. According to the Examiner, the specification does not describe a method of solid phase synthesis for preparing a LPA having undefined amino acid sequences between 4-20 amino acids in length and does not disclose that any achiral dicarboxylic acid can be employed. The Examiner states further that it is not clear as to the kind of amino acids or combinations thereof that can be synthesized, and that there may exist problems with longer amino

acid chains, and that Applicants have not sufficiently described the correlation between the specific examples recited in Applicants' application and the broad genus claimed in claims 84-86. Applicants respectfully traverse this rejection.

First, Applicants submit that the claims as presented are substantially limited to the use of simple achiral dicarboxylic acids as the bridging group. The general applicability of these acids is illustrated with imino diacetic acid (Examples 1, 2 and 6), 3-amino glutaric acid (Examples 3, 4 and 5), glutaric acid (Examples 7, 9. 10 and 11) and tricarballylic acid (Example 8). Please note that although tricarballylic acid is in fact a tricarboxylic acid, it is used as a dicarboxylic acid, see e.g. Example 8, wherein the surplus carboxy group is available for subsequent coupling.

Second, the Examiner is taking the position that it is not routine for those of ordinary skill in the art to be able to synthesize peptides up to 20 amino acids in length. As such, the examples provided in Applicants' specification are not sufficient to enable a genus of any 4-20 amino acid LPA. Applicants do not agree with the Examiner's characterization of the art, or the scope of Applicants' claims.

Claim 84 is directed to a method of making LPAs having between 4-20 amino acids. Applicants submit that it was routine at the time of filing the original application (September 29, 1998), to make peptides using solid phase synthesis techniques of 4-20 amino acids in length, regardless of the sequence. Applicants state that even longer peptides, of 40 or more amino acids, were well within the scope of those of ordinary skill at the time of

filing. Applicant invites the Examiner to review copies of abstracts of published scientific literature found in PubMed, which are directed to creation of various peptides using solid phase synthesis and attached collectively hereto as Attachment B.

In Attachment B, Applicants provide abstracts of prior art papers dating back to 1984, showing that synthetic peptides having more than 20 amino acids were well within the scope of those skilled in the art. Examples of synthesized peptides include human growth hormone (hGH, 29 amino acids), vasoactive intestinal peptide (VIP, 31 amino acids), follicle stimulating hormone (FSH, 48 amino acids), and chaperonin 10 (101 amino acids). These references clearly show that 4-20 amino acid peptides are not problematic and, in fact, were routinely prepared in peptide synthesis machines, at the time Applicants' application was filed.

Furthermore, the state of the art has progressed to where peptide synthesizers easily make 100 amino acid peptides of any sequence. See, for example, the sales brochure of Applied Biosystems, Inc.'s (Foster City, CA) peptide synthesizing machine, the 433A, which is attached hereto as Attachment C. Applicants obtained the brochure from the company website. Pages 4 and 5 of the brochure clearly show that the machine is easily capable of synthesizing very long peptides.

Furthermore, Applicants submit that the general applicability of the method of the invention for preparing LPA for presentation of peptide sequences has been illustrated for a wide number of sequences from different sources, for example, Borrelia burgdorferi (Examples 1-5), Mycobacterium tuberculosis (Examples 5-6), Chlamydia trachomatis (Examples 7-8 and Chlostridium

thermosacchrolyticum (Example 12), as well as sequences derived from angiotensin-I (Examples 9-12).

Accordingly, no undue experimentation is necessary and only routine synthetic methods known in the art are necessary to prepare LPAs having any amino acid sequence from 4 to 20 amino acids in length. Therefore, Applicants respectfully submit that the claims as pending are enabled and the rejection under 35 U.S.C. 112, first paragraph, should properly be withdrawn.

Rejections under 35 U.S.C. §102(b)

The Examiner has rejected claims 84-86 under 35 U.S.C. §102(b) as anticipated by Bhatnagar et al. (J. Med. Chem. 1996, 39, 3814-3819). The Examiner offers Bhatnagar et al. for teaching a method for solid phase synthesis of a dimer using a 0.5 mole equivalents diaminocarboxylic acid, thereby anticipating the claims. Applicants traverse this rejection.

Bhatnagar et al. teach a method for making a dimer peptide by synthesizing the peptide using solid phase synthesis and then cleaving the peptides from the resin. Then Bhatnagar et al. use diaminosuberic or diaminoadipic acid, which are chiral acids, to couple two peptide chains together in the middle of the peptide chains. This method and product is completely different than what is taught and claimed by Applicants' application. Applicants use only achiral di, or tri carboxylic acids to avoid racemization, and the cyclization and attachment of the peptide chains of Applicants' invention are performed while the peptides are still attached to the synthesis resin, not after cleavage as in Bhatnagar et al. Therefore, Bhatnagar et al. do not teach all the elements of

Applicants' claimed invention and therefore cannot anticipate the claims. Applicants request withdrawal of this rejection.

The Examiner rejected claims 84-86 under 35 U.S.C. §102(b) as anticipated by Lange et al. (J. Pep. Sci., 4:289-29). According to the Examiner, Lange et al. disclose a method of solid phase synthesis of dimeric peptide bradykinin using a diaminocarboxylic acid linker. As such, the Examiner states that the teaching fully meets Applicants' claimed method. Applicants traverse this rejection.

Like Bhatnagar et al., Lange et al. teach a method of making a bradykinin dimer using chiral diaminocarboxylic acids, such as diaminosuberic acid or diaminosebacic acid (page 290). In contrast to the N-terminal linkage of Applicants' LPAs, the peptides of Lange et al. are internally linked using various spacers, in a reaction which is completely distinct from Applicants' claimed method. Moreover, the dimers created in Lange et al. are N-terminal presenting, unlike Applicants', which are C-terminal presenting. Therefore, Lange et al. do not teach all the elements of Applicants' claimed invention and therefore cannot anticipate the claims. Applicants request withdrawal of this rejection.

The Examiner also rejected claims 84-86 under 35 U.S.C. §102(b) as anticipated by Alberts et al. (Peptides, p. 367-369). The Examiner states that Alberts et al. appear to teach synthesis of a dipeptide employing a dicarboxylic acid and therefore anticipates Applicants' claimed invention. Applicants traverse this rejection.

Applicants submit that Alberts et al. teach a method for making a dipeptide which is substantially the same as the method and product taught in either Bhatnagar et al., or Lange et al. Alberts et al. also teach the synthesis of a dipeptide having an internal sequence linkage, like Lange et al., using a chiral diaminocarboxylic acid. This method of synthesis, and the resulting product, are completely distinct from Applicants' claimed method. Therefore, Alberts et al. do not teach all the elements of Applicants' claimed invention and therefore cannot anticipate the claims. Applicants request withdrawal of this rejection.

Rejections under 35 U.S.C. §103(a)

The Examiner rejected claims 84-93 under 35 U.S.C. §103(a) as unpatentable over either Bhatnagar et al. or Lange et al., in view of Mathiesen et al. (WO 97/422210). The Examiner states that neither Bhatnagar et al. nor Lange et al. teach applying the solid phase synthesis method to a peptide derived from the OspC peptide of Borrelia burgforferi. The Examiner offers Mathiesen et al. for disclosing a method of making a peptide from a sequence of the OspC peptide of Borrelia burgforferi having the sequence of SEQ ID NO:1, by solid phase synthesis. The Examiner argues that it would have been obvious to one of ordinary skill in the art, at the time the invention was made, to synthesize a peptide using the method of Bhatnagar et al. or Lange et al. to make the dimer peptides of Mathiesen et al. to generate greater immune responses to the OspC peptide of Borrelia burgforferi. Applicants traverse this rejection.

The burden is on the Examiner to establish a *prima facie* case of obviousness of the claimed subject matter over prior art

references. In re Deuel, 51 F.3d 1552, 1557, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995). Only after that burden is met must the applicant come forward with arguments or evidence in rebuttal. Id. To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

Applicants respectfully submit that Bhatnagar et al. and/or Lange et al. fail to teach a method of solid phase synthesis of a ligand presenting assembly (LPA) where the peptides are synthesized such that they create a dipeptide being connected by an achiral carboxylic acid at their N-terminus, and have free C-terminal ends. The methods of synthesis and the resulting products of both Bhatnagar et al. and Lange et al. are completely distinct from Applicants' claimed method. This failure is not cured by the addition of the teachings of the Borrelia sequences in the Mathiesen et al. reference. The combination of Bhatnagar et al. or Lange et al., in view of Mathiesen et al., does not teach each and every element of Applicants' claimed invention. Thus, Applicants respectfully request withdrawal of this rejection

No new matter has been added by this Response. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,
JACOBSON HOLMAN PLLC

By _____
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SN: 10/724,233
DKT: P63882US1
INV: Holm et al.

LPA. Background and overwiev

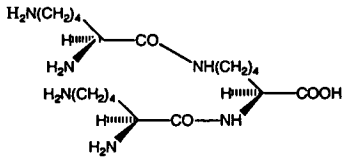
Figure 1

H_2N-Pro-Val-Val-Ala-Glu-Ser-Pro-Lys-Lys-Pro-COOH

Antigenic OspC C-terminal sequence

] Prior Art

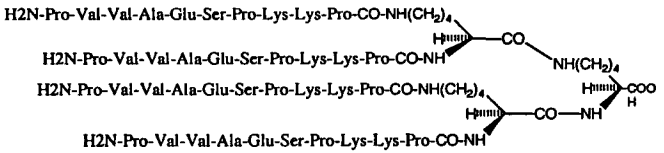
Figure 2



"Lysine tree" for *multiple N-terminal presentation* of antigenic sequence: MAP (Multiple Antigen Peptide (J.P.Tam)) for enhancement of peptide immunogens.

] Prior Art

Figure 3



A MAP system shown with antigenic OspC sequence

] Prior Art

Figure 4

A *multiple N-terminal presentation cannot* be used for the OspC sequence. because Ab´s (antibodies) have a high C-terminal affinity towards

H_2N-Pro-Val-Val-Ala-Glu-Ser-Pro-Lys-Lys-Pro-**COOH**

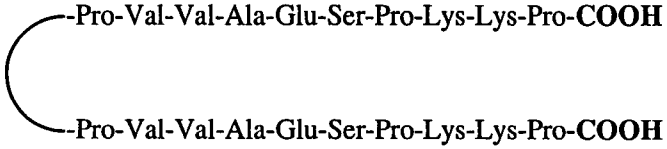
but low affinity towards

H_2N-Pro-Val-Val-Ala-Glu-Ser-Pro-Lys-Lys-Pro-**$CONH_2$**

(a MAP system has a **CONH**-bond)

Problem Being Solved

Figure 5

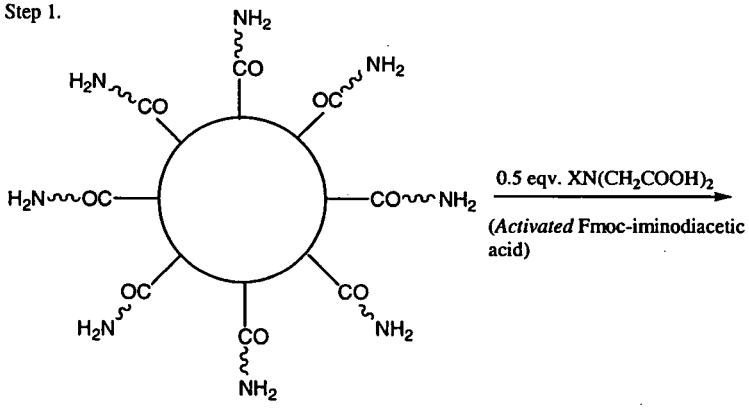


A needed system for multimeric (dimeric) C-terminal (COOH) presentation based on linked amino groups.

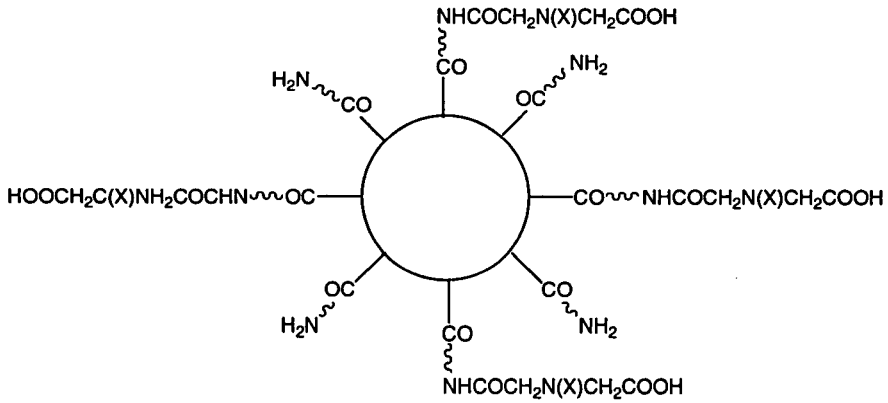
INVENTION

Figure 6

Acylation. Step 1.



The drawing depicts a single resin bead (out of a large number) with a number of identical preformed fully side-chain protected peptide chains having a free N-terminal amino group.

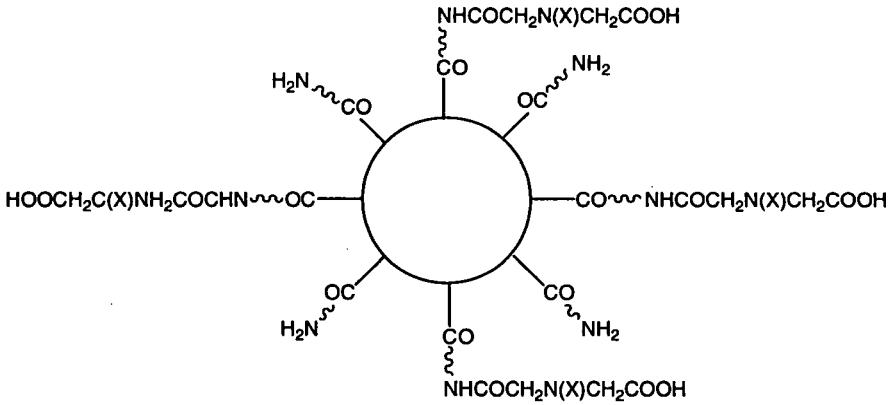


1/1 formation of acylated and un-acylated peptide chains

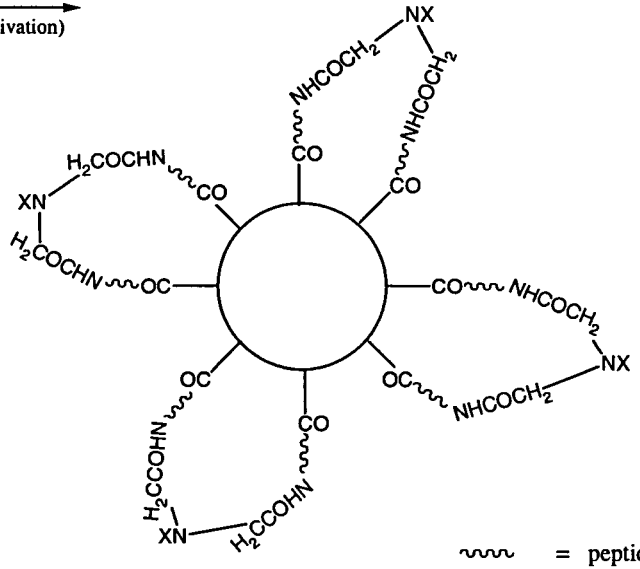
~~~ = peptide sequence

X = Fmoc-protection
~~~

Figure 7

Acylation. Step 2.



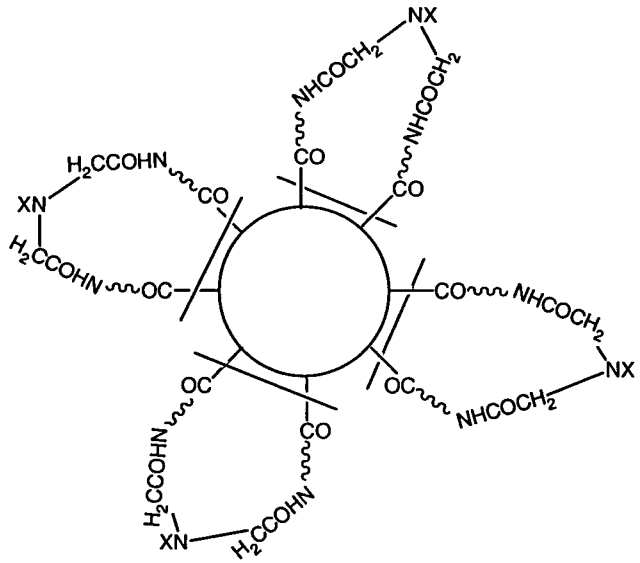
(Activation)



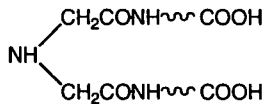
~~~ = peptide sequence

Activation is carried out using standard peptide synthesis methods.
~~~

Figure 8

Acylation. Cleavage to give LPA.



Standard Fmoc-deprotection and standard cleavage from the resin withTFA/scavengers



LPA with iminodiacetic acid

X = Fmoc-protection

∿∿∿ = peptide sequence

—— = bond cleavage

1: Naturwissenschaften. 1984 May;71(5):252-8.

Links

[Peptide chemistry today]

[Article in German]

Geiger R.

Peptide chemistry has reached a plateau on which more work is devoted to the improvement of known basic principles than to the development of really new methods. As a consequence, routine syntheses of peptides concentrate on few protecting groups and coupling procedures only. The recent progress in purification of peptides by liquid chromatography helped to promote this trend. Meanwhile, recombinant technology permits the synthesis of proteins and possibly of some lower peptides, too. It cannot compete, however, with chemical solid-phase synthesis of a newly discovered peptide with up to about 40 amino acids in speed but may take over the synthesis for large-scale production. Peptides containing modified or non-natural structures, which are often superior to the natural compounds as drugs, will remain subject of chemical synthesis, too. Present research focuses on the role of peptide and protein factors in the immune response and on structure and biological activities of neuropeptides.

PMID: 6087164 [PubMed - indexed for MEDLINE]

Related Links

Concept and early development of solid-phase peptide synthesis. [Methods Enzymol. 1997]

[Solid-phase synthesis of peptides modelling the transmembrane segment of bacteriorhodopsin] [Biochimie. 1993]

[Side reactions in peptide synthesis. V. O-sulfonation of serine and threonine during removal of pmc- and mtr-protecting groups from arginine residues in fmoc-solid phase synthesis] [Biol Chem Hoppe Seyler. 1993]

Semisynthetic peptides and proteins. [CRC Crit Rev Biochem. 1981]

Solution syntheses of two enkephalin-containing peptides, peptide E and dynorphin(1-24), using Nin-(2,4,6-triisopropylphenylsulfonyl) tryptophan. [Chem Pharm Bull (Tokyo). 1989]

See all Related Articles...

1: J Pept Res. 1998 Aug;52(2):155-64.

Combined solid-phase/solution synthesis of a 31-residue vasoactive intestinal peptide analog: general method for repetitive coupling of fragments without isolation and purification of intermediates.

Felix AM, Zhao Z, Lambros T, Ahmad M, Liu W, Daniewski A, Michalewsky J, Heimer EP.

Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey, USA. FelixAssoc@aol.com

A novel analog of vasoactive intestinal peptide (VIP) has been reported which exhibits high potency and enhanced duration of in vivo biological activity. This VIP analog, cyclo-(Lys21-Asp25)Ac[Glu8 Lys12 Nle17 Ala19, Asp25 Leu26,Lys27,28,Gly29,30,Thr31]-VIP, which also has a lactam bridge, has been reported to have relaxant effects that are significantly more potent than other beta-agonists such as salbutamol and salmeterol. Because it has potential use for the treatment of bronchial asthma in humans, various convergent syntheses were evaluated to enable the economic preparation of large quantities of this medium-sized hentriacontapeptide. From these studies we developed a combined solid-phase/solution synthesis which uses four protected fragments (each prepared by solid-phase synthesis with highly acid-labile resins) possessing Nalpha-9-fluorenylmethyloxycarbonyl and side-chain tert-butyl protection. Only equivalent amounts of each fragment were required to achieve near-quantitative coupling reactions using N-[(1H-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmeth anaminium hexafluorophosphate N-oxide/N-hydroxybenzotriazole. All reagents and side products were removed at each stage by simple extraction procedures. Final deprotection was carried out with 90% trifluoroacetic acid. Under these conditions only low levels of epimerization were observed (<2%). These diastereoisomers and other trace impurities were removed from the product in a single purification by preparative high-performance liquid chromatography. The procedure has been scaled up (10-g scale) and the final product obtained in an overall (nonoptimized) yield of 24%. This procedure for the repetitive coupling of fragments, without isolation of intermediates, may be generally applicable for the economic synthesis of other medium-sized and longer peptides.

PMID: 9727872 [PubMed - indexed for MEDLINE]

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Nov 6 2006 15:24:20

1: J Pept Res. 1998 Feb;51(2):134-41.

Links

New analogs of human growth hormone-releasing hormone (1-29) with high and prolonged antagonistic activity.

Toth K, Kovacs M, Zarandi M, Halmos G, Groot K, Nagy A, Kele Z, Schally AV.

Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70146, USA.

Based on our previous results, in conjunction with various structural considerations, 19 new analogs of the GHRH antagonist [PhAc-Tyr1,D-Arg2,Phe(pCl) 6,Abu15,Nle27,Agm29]++ +hGHRH(1-29) (MZ-5-156) were synthesized by the solid-phase method. These compounds were designed to develop further analogs of this class with increased receptor-binding affinity. All analogs had Abu15 and Nle27 modifications and were acylated with phenylacetic acid at the N-terminus. Most of the analogs had D-Arg2 and Phe (pCl)6 substituents and Agm29 or Arg29-NH2 at the C-terminus. Additional single substitutions consisted of the incorporation of D- or L-Tic1, D-Tic2, Tic6 or Phe(pNO2)6 and Arg29-NH2. The Arg29-NH2 analog of MZ-5-156 (KT-48) was further modified by single substitutions using Pal1; D-Tpi2; D- or L-Phe4; Phe(pX)6 X = F, Cl, I; Tyr7; Aib8; Tyr(Me)10 or Phe(pCl)10. Four peptides had multiple substitutions. All the analogs were evaluated for their ability to inhibit GH release induced by hGHRH(1-29)NH2 in vitro and some were also tested in vivo. Peptides [PhAc-Tyr1,D-Arg2,Phe(pI) 6,Abu15,Nle27]hGHRH(1-2 9)NH2 (KT-30), [PhAc-Tyr1,D-Arg2,Phe(pCl)6,Aib8,Abu15,Nle27] hGHRH(1-29)NH2 (KT-50) and [PhAc-Tyr1,D-Arg2,Phe(pCl)6,Tyr(Me)10,Abu15,Nle27]h GHRH(1-29)NH2 (KT-40) with Phe(pI)6, Aib8 or Tyr(Me)10 modifications, respectively, showed high and prolonged inhibitory effect in superfused rat pituitary system. Analog KT-50 also exhibited a strong and long-term inhibitory activity in vivo in rats. Most of the new analogs showed high binding affinities to rat pituitary GHRH receptors.

PMID: 9516049 [PubMed - indexed for MEDLINE]

Nov 6 2006 15:24:20

1: J Pept Sci. 1997 Nov-Dec;3(6):397-414.

Links

Synthetic hFSH peptide constructs in the evaluation of previous studies on the hFSH receptor interaction.

Spetzler JC, Meldal M, Meinjohanns E, Steinaa L, Mouritsen S, Bock K.

Department of Chemistry, The Carlsberg Laboratory, Valby, Denmark.

The human follicle-stimulating hormone (hFSH) belongs to a family of glycoprotein hormones which contains two non-identical subunits. This paper describes the design and synthesis of a series of synthetic hFSH constructs as putative ligands for the receptor. The design of these constructs is based on the crystal structure of hCG and molecular modelling using the program package Insight II/Discover. The designed constructs contain peptides ranging from 7 to 48 amino acid residues, disulphide bridges and glycan residues. All the synthetic peptides were synthesized by the stepwise solid-phase method using Fmoc chemistry. Two of the synthetic peptides contain the glycosylated amino acid. Asn (GlcNAc-GlcNAc) and both were prepared using fully protected glycosylated building blocks in the solid-phase peptide synthesis. The disulphide bridges were formed from acetamidomethyl-protected glycopeptides and peptides by a direct deprotection/oxidation method using thallium(III) trifluoroacetate. Mass spectroscopy and amino acid analysis were used for characterization of the synthetic hFSH glycopeptides and peptides. The synthetic hFSH constructs were tested for binding activity on FSH receptor assays but none showed improved binding properties compared with the naturally occurring hormone. It was finally demonstrated that non-related peptides showed non-specific binding at the same level as reported for specific peptides.

PMID: 9467969 [PubMed - indexed for MEDLINE]

Nov 6 2006 15:24:20

1: Int J Pept Protein Res. 1996 Jul;48(1):31-47.

Links

Chemical synthesis and purification of proteins: a methodology.

Ball HL, **Mascagni P**.

Department of Peptide Chemistry, Italfarmaco Research Centre, Cinisello Balsamo, Milan, Italy.

Classical stepwise solid-phase peptide synthesis (SPPS) has been used successfully for the synthesis of proteins up to 150 residues in length, although usually with poor yields and homogeneity. The major limitation has been the inability to separate chromatographically similar deletion and truncated impurities from the target sequence. We have developed a highly effective protocol for stepwise SPPS and 'one-step' purification of small proteins. to demonstrate the effectiveness of the methodology we synthesised the 101 residue chaperonin 10 protein from Rattus norvegicus (Rat Cpn 10) using three different chemical protocols. Highly homogeneous Rat Cpn10 was obtained using an optimised synthetic strategy and one-step purification procedure (method C), involving (i) HBTU/HOBt activation, (ii) N-(2-chlorobenzyloxycarbonyloxy)succinimide as capping agent and (iii) the incorporation of a reversible Fmoc-based chromatographic probe, derivatised with a lipophilic group for fast one-step RP purification, to give an overall yield of 9.6%. Analysis by ESI-MS indicated that the product was virtually free of deletion impurities, while RP-HPLC under four different conditions and CZE indicated that the protein was 100 and 84% pure, respectively. The spontaneous folding of Rat Cpn10 into its biologically active form was found to correlate well with the degree of purity as assessed by chromatography, ESI-MS and sequencing, since 29 (A), 55 (B) and 81% (C) of correctly folded heptameric structure was obtained. The degree of homogeneity was also reflected in the ability of purified Rat Cpn10 to facilitate the refolding of yeast enolase.

PMID: 8844261 [PubMed - indexed for MEDLINE]

Nov 6 2006 15:24:20

1: J Biol Chem. 1996 Jun 14;271(24):14421-9.

Covalent structure, synthesis, and structure-function studies of mesentericin Y 105(37), a defensive peptide from gram-positive bacteria Leuconostoc mesenteroides.

Fleury Y, Dayem MA, Montagne JJ, Chaboisseau E, Le Caer JP, Nicolas P, Delfour A.

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A 37-residue cationic antimicrobial peptide named mesentericin Y 105(37) was purified to homogeneity from cell-free culture supernatant of the Gram-positive bacterium Leuconostoc mesenteroides. The complete amino acid sequence of the peptide, KYYGNGVHCTKSGCSVNWGEAASAGIHRLANGGNGFW, has been established by automated Edman degradation, mass spectrometry, and solid phase synthesis. Mesentericin Y 105 (37) contains a single intramolecular disulfide bond that forms a 6-membered ring within the molecule. Mesentericin Y 105 (37) was synthesized by the solid phase method. The synthetic replicate was shown to be indistinguishable from the natural peptide with respect to electrophoretic and chromatographic properties, mass spectrometry analysis, automated amino acid sequence determination, and antimicrobial properties. At nanomolar concentrations, synthetic mesentericin Y 105(37) is active against Gram+ bacteria in the genera Lactobacillus and Carnobacterium. Most interestingly, the peptide is inhibitory to the growth of the food-borne pathogen Listeria. CD spectra of mesentericin Y 105(37) in low polarity medium, which mimic the lipophilicity of the membrane of target organisms, indicated 30-40% alpha-helical conformation, and predictions of secondary structure suggested that the peptide can be configured as an amphipathic helix spanning over residues 17-31. To reveal the molecular basis of the specificity of mesentericin Y 105 (37) targetting and mode of action, NH2- or COOH-terminally truncated analogs together with point-substituted analogs were synthesized and evaluated for their ability to inhibit the growth of Listeria ivanovii. In sharp contrast with broad spectrum alpha-helical antimicrobial peptides from vertebrate animals, which can be shortened to 14-18 residues without deleterious effect on potency, molecular elements responsible

for anti-Listeria activity of mesentericin Y 105(37) are to be traced at once to the NH2-terminal tripeptide KYY, the disulfide bridge, the putative alpha-helical domain 17-31, and the COOH-terminal tryptophan residue of the molecule. It is proposed that the amphipathic helical domain of the peptide interacts with lipid bilayers, leading subsequently to alteration of the membrane functions, whereas residues 1-14 form part of a recognition structure for a membrane-bound receptor, which may be critical for peptide targetting. Because mesentericin Y 105(37) is easy to synthesize at low cost, it may represent a useful and tractable tool as a starting point for the design of more potent analogs that may be of potential applicability in foods preservation.

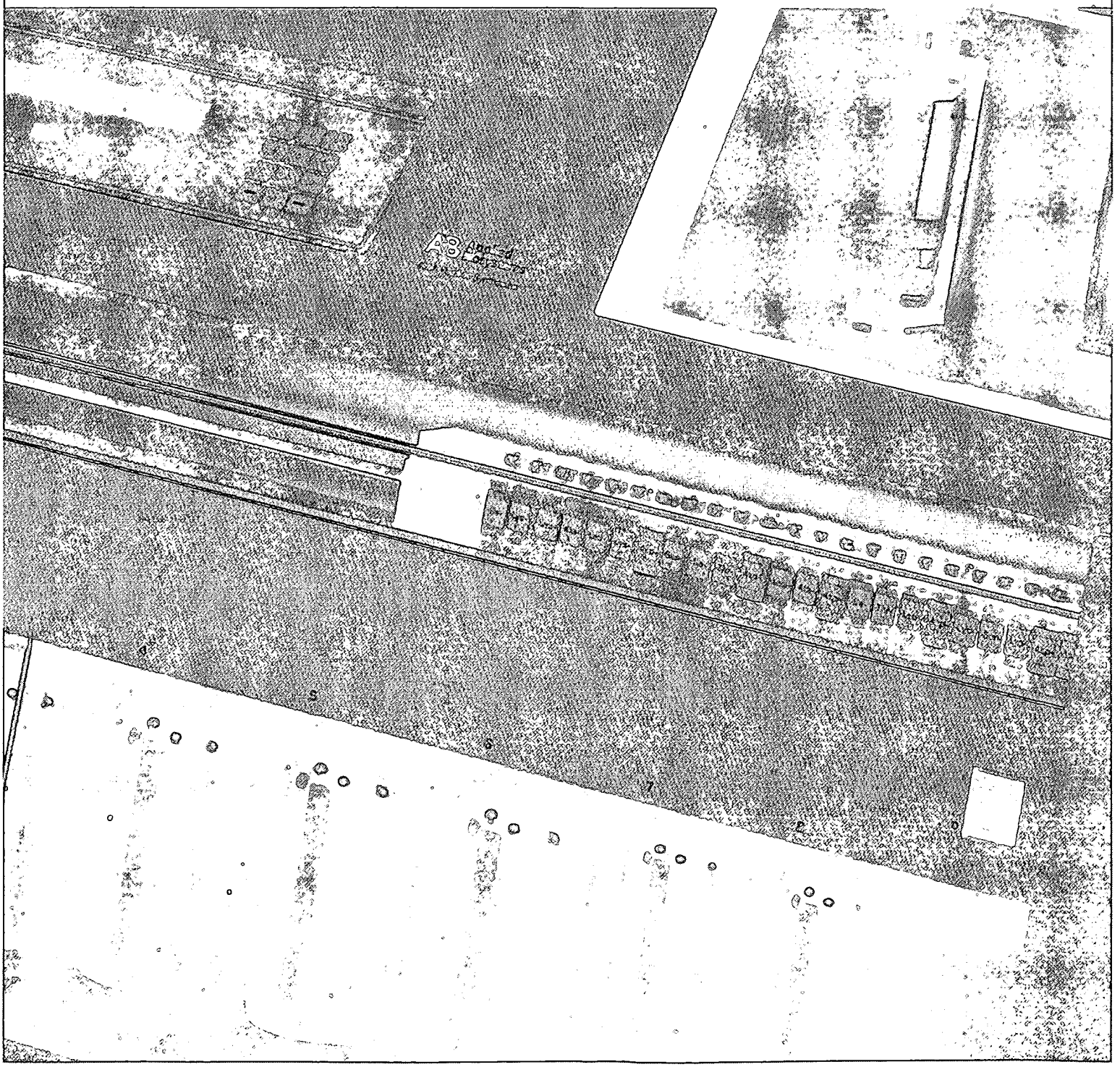
PMID: 8662868 [PubMed - indexed for MEDLINE]

Meeting Advanced Peptide Research Demands

Applied Biosyste

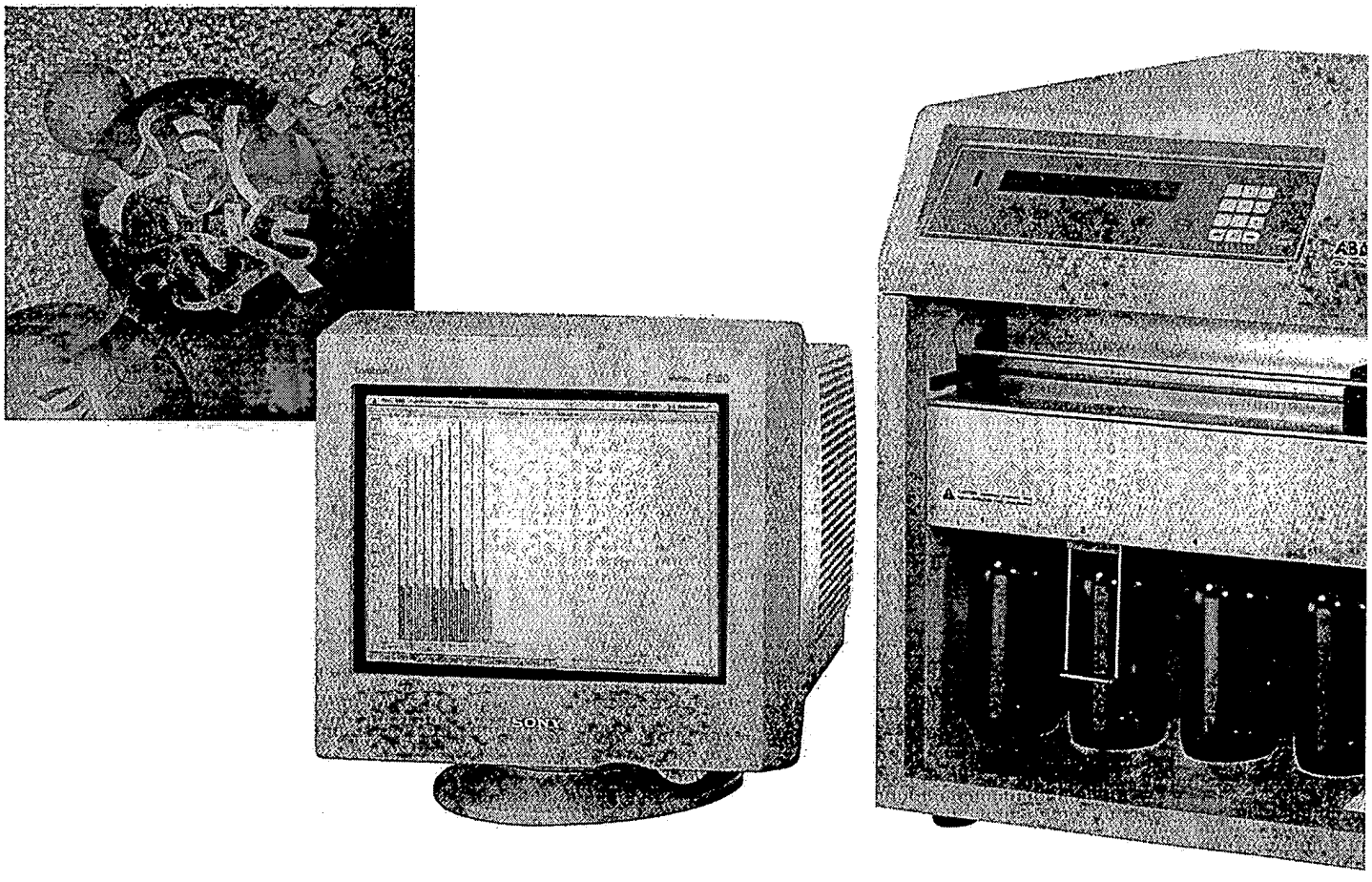
433A

Peptide Synthesis System



The gold standard of peptide synthesis

The 433A peptide synthesizer from Applied Biosystems is the gold standard of performance in peptide synthesis. It offers an extraordinary range of features that provide efficiency, functionality, and versatility. The 433A peptide synthesizer has the largest installed base of any peptide synthesis system because of its proven history of superior performance.



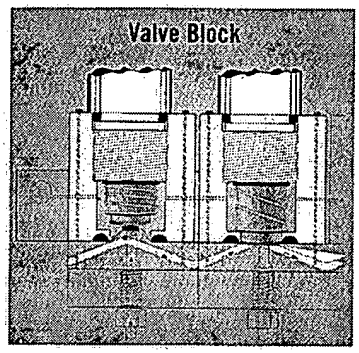
Proven Flexibility

Proven Results

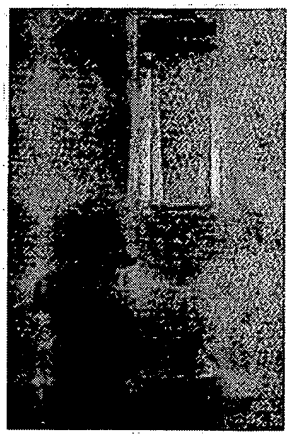
Proven Quality

Proven Performance

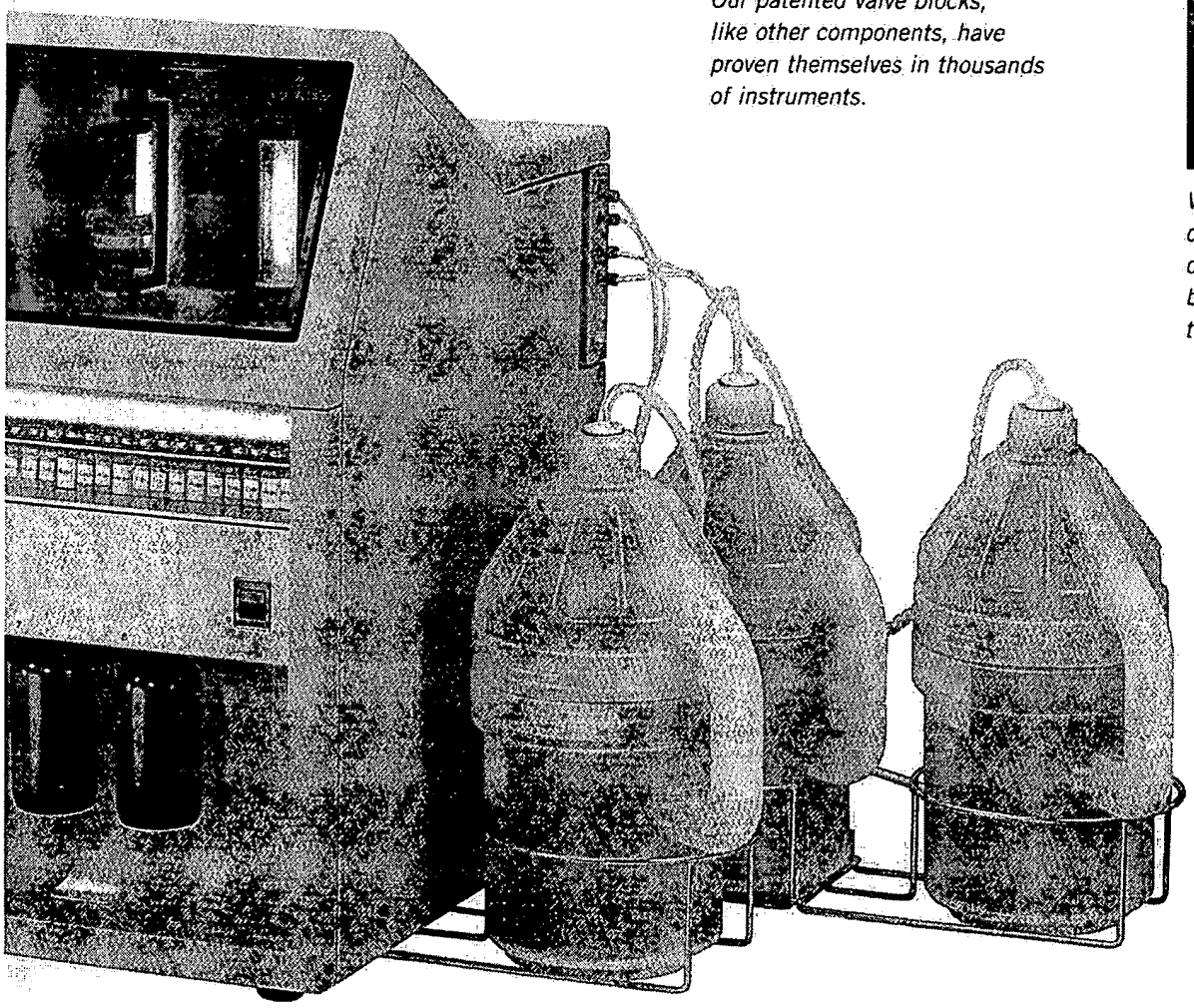
Tance



Our patented valve blocks, like other components, have proven themselves in thousands of instruments.



Vortex mixing ensures continuous, highly concentrated interactions between the peptide and the activated amino acid.



Uncompromising chemistries, unrivaled flexibility

Synthesize long and complex peptides

No Compromises

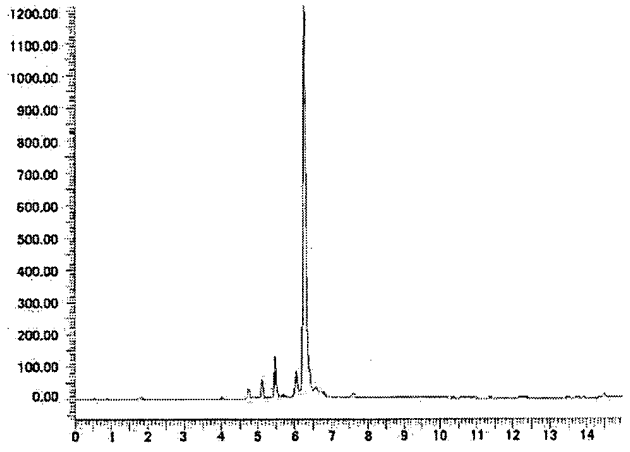
Advanced peptide research demands uncompromising instrumentation and flexible chemistry. With the 433A, you can choose tBoc or Fmoc chemistry—or switch between them. If you need long, complex peptides, or have other special synthesis needs, you can employ a variety of chemistry strategies to obtain optimal results.

Benefits include:

- Wide range of peptide synthesis scales from 0.10 to 1.0 mmoles, and optional scales with the 3-mL reaction vessel of 5, 10, or 20-µmole.
- Separate activation vessel permits any activation strategy—preactivated, or *in situ*—while also saving time. As one residue undergoes coupling in the reaction vessel, the next residue is simultaneously activated. You can use any carbodiimide, uronium or other activator.
- Flexibility to utilize any type of resin.
- Engineering design that accommodates trifluoroacetic acid, a highly corrosive liquid. The 433A valve blocks have proven their reliability in thousands of instruments.
- Capability to synthesize complex or long sequences, e.g. 100-mer peptides.

3mL Reaction Vessel Kit

Lets you perform very small-scale synthesis with the 433A. This smaller reaction vessel accommodates 5, 10, and 20-µmol-scale synthesis. These lower scales let you economically use peptide nucleic acid (PNA) monomers, glycosylated amino acids, and isotopically labeled amino acids.

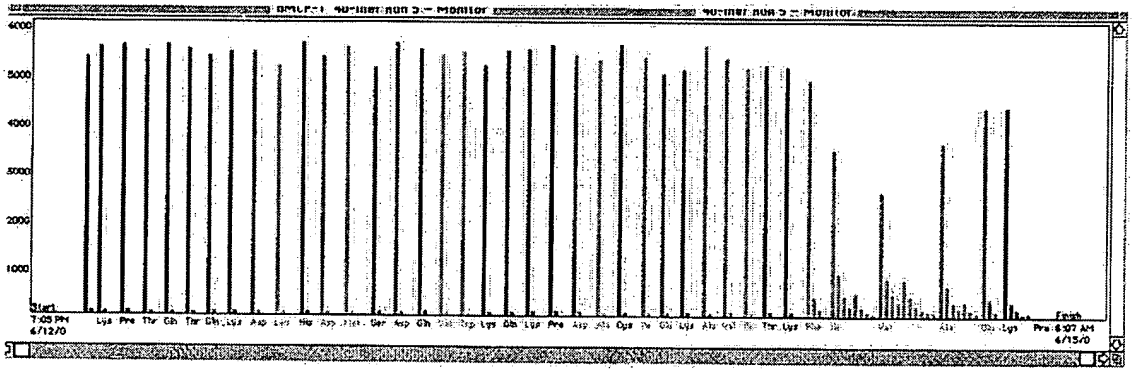


PNA sequence of H-TCA-TAG-ACA-ATT-NH_2 synthesized to 83% purity by integration.

Achieve better results syntheses

Faster synthesis, superior peptides

Monitoring with feedback control



Histogram data for a 40-residue peptide synthesizing using feedback control to extend deprotection and coupling for difficult cycles.

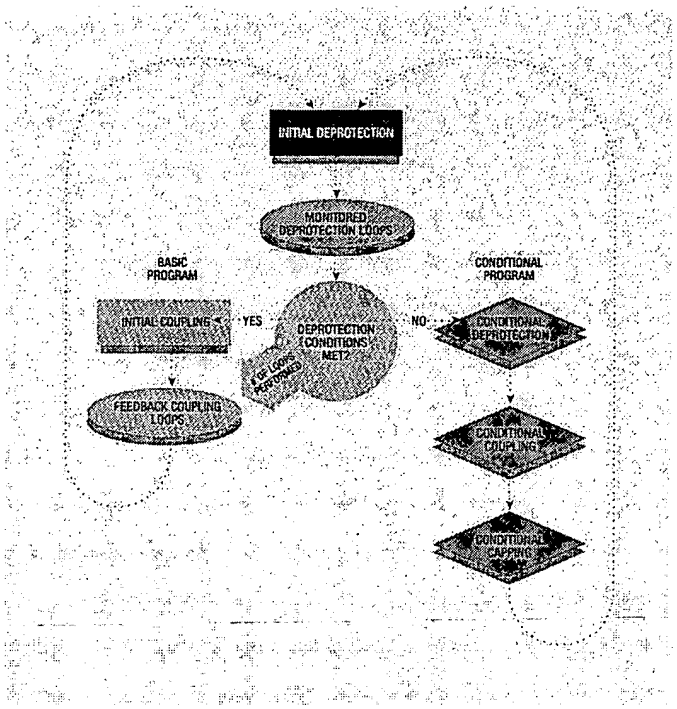
At the heart of the 433A is a unique feedback monitoring system that allows you to create longer, more complex peptides-efficiently. The monitoring system monitors the deprotection reactions and automatically extends the deprotection and coupling in difficult regions. This enables you to improve throughput by programming short cycle times, and rely on the instrument to lengthen a cycle only for difficult couplings. The result: faster synthesis, greater coupling yields, and superior peptides. You save time and reduce reagent waste.

Monitoring choices to meet your needs

Two monitoring programs are available to meet your research and information needs. These programs direct instrument operations based on the information obtained from the 433A monitoring system.

The flow diagram illustrates both programming options. With the basic control program (left side of the diagram) when the deprotection percentage is achieved, the 433A proceeds to initial coupling. The 433A counts the number of monitoring deprotection loops performed to achieve your required deprotection. When the synthesis proceeds to coupling, the 433A performs the same number of feedback coupling loops before advancing to the next cycle.

For more difficult peptides, the conditional capability lets you program the synthesizer to perform more complex operations based on the monitoring data. The conditional program directs the synthesizer to operate according to your instructions—if the deprotection conditions are not met (right side of the diagram). As the diagram shows, additional deprotection can be followed by coupling and capping steps before the system proceeds to the next cycle. You can also develop your own deprotection program. Other choices could include double coupling, changing coupling condition with co-solvents or additives, or stopping the synthesizer.



With conditional programming, you do not have to treat an entire peptide as difficult just because of a few troublesome regions. Instead, you can plan ahead for potentially difficult coupling without having to predict exactly where the problems will occur.

Intuitive programming

The SynthAssist Software and the computer included with the 433A offers powerful programmability and control. This system provides the power to take full advantage of the 433A's innovative feedback control technology. Designed specifically for peptide synthesis, this intuitive software:

- Assists in rapidly setting up every aspect of a run.
- Collects and stores data, including a log of everything that occurs during your synthesis. It stores files for reference, repeat runs or future modifications.
- Analyzes results.
- Calculates coupling yields, molecular weight and amino acids composition.
- Stores information regarding the compounds and reagents you normally use in a dictionary file. You can add more, such as unique amino acids, novel protecting groups and compounds developed in the future.
- Offers chemistry files that are preprogrammed for the full range of synthesis scales, from 0.1 to 1.0 mmole. Run them as is or use them as starting frameworks you can modify to suit your unique needs.
- Allows quick and easy editing of sequences and synthesis files to fine-tune customer runs. You can mix and match functions, steps, modules and cycles to create the exact synthesis run you need.

"Our AB 433A instruments are the firm basis of our peptide synthesis unit. The ease of application, speed and reliability make them especially suited for special syntheses including long and difficult peptides."

Holger Wenschuh, PhD.
Head of Jerini Peptide Technologies
Jerini AG

results

Synthesis of long peptides on the 433A

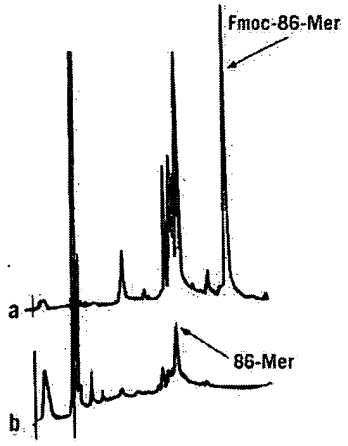
The synthesis of long peptides is of interest because functionally active protein and enzymes are roughly 100 amino acids in length. The stepwise automated synthesis of long peptides, 75–100 amino acids or more, can readily be carried out on the 433A peptide synthesizer.

The preparation of TAT 86-mer, the translational activating protein from the RNA viruses, is shown below.

Fmoc-Met-Glu-Pro-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-Gln-Pro-Lys-Thr-Ala-Cys(Acm)-Thr-Thr-Cys(Acm)-Tyr-Cys(Acm)-Lys-Lys-Cys(Acm)-Cys(Acm)-Phe-His-Cys(Acm)-Gln-Val-Cys(Acm)-Phe-Thr-Thr-Lys-Ala-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Pro-Pro-Gln-Gly-Ser-Gln-Thr-His-Gln-Val-Ser-Leu-Ser-Lys-Gln-Pro-Thr-Ser-Gln-Pro-Arg-Gly-Asp-Pro-Thr-Gly-Pro-Lys-Glu-OH

The 86-mer TAT sequence. The seven cysteine residues in the sequence were incorporated as the Acm derivative for both synthesis and analysis.

The synthesis of the TAT 86-mer was carried out on the 433A using standard reagent for *FastMoc* chemistry, UV monitoring of the Fmoc deprotections, and conditional chemistry run files. The conditional chemistry files automatically extended deprotections, extended couplings, and then capped each cycle whenever the deprotections failed to reach a baseline level rapidly.



HPLC results of the Fmoc protected (a) and the unprotected (b) 86-mer TAT sequence product.

"If you want the best peptide synthesizer for large peptides, there is no doubt that the 433A is the choice."

David Schooley
Professor of Biochemistry and Director of the Protein Microanalysis and Mass Spectrometry Facilities
University of Nevada

from our lab to yours

Dependable and collaborative support

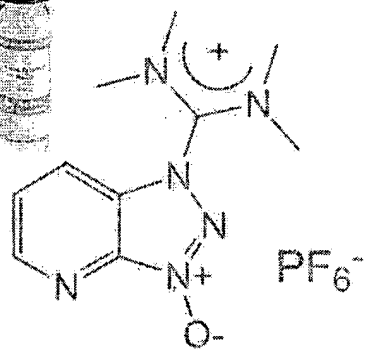
Applied Biosystems has been a leader in peptide synthesis for two decades. We continue to provide technical innovation, reliable instrumentation, and ongoing application development to make it easy for you to synthesize the high-quality peptides you need.

Service, technical support and field applications

We are committed to helping our customers break new ground. Our service ensures the smooth day-to-day operation of your system. We provide strong application and technical knowledge, resulting in unequalled customer support.

Chemistry

We are dedicated to providing innovative technologies and products. We are leaders in the development of novel technologies, such as PEG-PS™ solid support, and high performance activation agents. We have strict quality control and pay close, careful attention to manufacturing processes to produce high-quality reagents.



"Applied Biosystems technical support has been top-notch."

Robin Daskin
Scientist II
Genencor International

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